Abstract: 49O

C18ORF25 is a novel exercise-regulated AMPK substrate regulating skeletal muscle function

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Exercise regulates a diverse array of phosphorylation networks which are thought to promote numerous health benefits. Functionally characterising these networks hold great promise in identifying new therapeutic targets for a range of diseases including type-2 diabetes, cancer and neurological disorders. Recently, we performed phosphoproteomic analysis of human skeletal muscles subject to endurance, sprint, and resistance exercise to identify canonical signalling pathways during and after exercise. This identified 5,486 phosphosites regulated during or after at least one type of exercise modality and only 420 core phosphosites common to all exercise. One of these core phosphosites was Ser-67 on the uncharacterized protein C18ORF25. Interestingly, integration with human genome-wide association studies linked genetic variants of C18ORF25 with glycated haemoglobin and type II diabetes.

The function of C18ORF25 is unknown but it shares sequence similarity with ARKadia (RNF111) which is associated with regulation of the TGF-beta/BMP pathway and is conserved in 99% of jawed vertebrates (Sriramachandran et al., 2019). To predict the upstream kinase(s) mediating phosphorylation of C18ORF25, we used a machine-learning approach which revealed Ser-67 lies within an AMPK consensus motif (Gwinn et al., 2008). Given the well described role of AMPK in metabolic adaptations during exercise, we hypothesise C18ORF25 is a novel regulator of exercise adaptations.

Here, we validate phosphorylation of Ser-67 on C18ORF25 as a novel exercise-regulated AMPK substrate. To characterise the functional role of C18ORF25, we generated a whole-body knockout (KO) mouse model. Our data reveal KO mice gained similar weight on a chow diet compared to wild type (WT) littermates, however, we observed a striking increase in adiposity and subtle decrease in lean mass from 6 weeks of age. Interestingly, KO mice on a chow or high-fat diet displayed no major differences in whole body glucose tolerance or skeletal muscle insulin sensitivity as assessed by *ex vivo* insulin-stimulated glucose uptake. Furthermore, forced treadmill exercise revealed KO mice fatigue quicker than WT mice. These data prompted us to further investigate skeletal muscle function revealing KO mice have significant reductions in *Soleus* force production compared to WT siblings. Histological analysis revealed no major difference in muscle fibre-type but a drastic reduction in fibre cross sectional area.

Moreover, proteomic analysis of *tibialis anterior* muscles from KO mice revealed increased extracellular matrix proteins including collagens, proteoglycans, glycosaminoglycans and elastic fibres. In contrast, loss of C18ORF25 resulted in a reduction of proteins associated with translation, pyruvate and branched-chain amino acid metabolism, NEDDylation and several mitochondrial metabolic pathways. Interestingly, the most significantly down-regulated protein in KO muscles was cAMP-dependent protein kinase catalytic subunit beta (PRKACB; also known as PKA-beta). Comparing our proteomic data to a recent transcriptomic/proteomic analysis of PKA KO epithelial cells showed an enrichment to those also down-regulated in PKA KO cells (Isobe et al., 2017) suggesting loss of C18ORF25 results in aberrant PKA-dependent signalling. Phosphoproteomic analysis of KO *soleus* muscles subject to *ex vivo* contraction revealed elevated phosphorylation of substrates downstream of MEK and LCK while substrates of PKA, ERK, MK2 and GSK3 displayed attenuated contraction-induced phosphorylation.

Taken together, our data suggest C18ORF25 plays a vital role in AMPK-mediated skeletal muscle adaptations to exercise and that loss of C18ORF25 attenuates several known exercise-induced signalling pathways and kinases including PKA that mediate skeletal muscle contractile function.

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